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(54) Title: TARGETING OF SPECIFIC CELL TYPES FOR REMOVAL BY THE IMMUNE SYSTEM

(57) Abstract

This disclosure provides a method of selecting one or more peptides or groups of peptide or protein bound to a carrier (for example, as part of peptide-expressing bacteriophage, from a peptide or protein expressing phage library or other ligand bound to an immunogenic carrier), that recognize and bind to epitopes exposed on one target class of cells or tissue only, and the application of the selected group of peptides or proteins or ligand and their carriers as markers to identify the desired target cells or target tissue *in situ*. Desirably, the immune system has been primed to recognize and respond to the carrier of the peptide or protein, so that a target cell/carrier/antibody complex is formed. Alternatively, the immune response to the carrier can be generated during treatment. By interposing the carrier between the and the responding antibody, the immune system's tolerance to the target is bypassed. Thus, the immune system can be made to target any desired cell within the body for elimination by decorating that target cell or target tissue with a carrier bearing a peptide which specifically binds to the target cell.

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TARGETING OF SPECIFIC CELL TYPES FOR REMOVAL BY THE IMMUNE SYSTEM

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority from United States Provisional Application Nos. 60/081,519, filed April 8, 1998 and 60/090,054, filed June 19, 1998.

BACKGROUND OF THE INVENTION

The field of this invention is immunology, especially as related to targeting of specific cell types for removal by the immune system.

Infected cells and tumor cells or tumor tissue are often either unrecognized by or escape attack by the immune system. This is thought to be because they do not express peptides, proteins or carbohydrates on their surface that are recognized as foreign or dangerous by the immune system, or that these cells do not respond differently from a normal cell or tissue, despite their expression of infection- or tumor-specific antigens on the cell surface. The present invention involves the process of selecting peptides or proteins that adhere to specific types of cells or to cell-specific surface molecules on or in infected or tumor cells, the production and amplification of the selected peptides or proteins that adhere to these small cellular differences, the use of the selected peptides or proteins to identify or mark the undesirable cells *in vivo* and the stimulation of the immune system to remove the marked target cells or target tissue from the body or to inhibit the growth of target cells or target tissue.

The self/non-self model of immunology has led to the assumption that a malignant cell's expression of characteristic proteins or poplypeptides is an expression of non-self and therefore should initiate an immune response against the malignant cell. The "danger" model of immunology predicts that normal tumor growth is not seen as dangerous by the immune system and therefore no response should be expected [Fuchs and Matzinger (1996) *Semin.*

Immunol. **8**:271]. An elaboration of the danger model implies that a new malignant cell continues dividing until some dangerous event occurs in the dividing cell's environment. Any event that is dangerous to a cell can potentially activate a neighboring dendritic cell and thus initiate an adaptive immune response. An activated dendritic cell will phagocytose material in its environment, including tumor proteins. There are three pathways for malignant tissue to develop immune tolerance. A naive T-cell with receptors (TCR) that recognized the characteristic tumor polypeptide in a MHCI complex, happens upon the malignant cell before the naïve T-cell has been activated by an activated antigen presenting cell (APC), the naïve T-cell would be deleted by engagement of MHCI signal only (no co-stimulatory signal). If a tumor is large and the danger is small, the first few times that the malignant cell mass encounters danger, peripheral tolerance should take over and turn off the T-cell response, just as any other self-reactive response would be turned off. Additionally, some tumors are able to develop other mechanisms for preventing a viable immune response. A tumor's very existence demonstrates some level of immune tolerization. The tumor cell's mechanism toward immune tolerance is probably similar though more elaborate than normal tissue control over immune tolerance mechanisms.

Breast cancers were treated by immune stimulation over 200 years ago by de Lisle [Fuchs and Matzinger (1996 supra] however, reproducibility has not been consistent. The cancer treatment disclosed herein functions by focusing the various parts of the immune cytotoxic and/or antibody and complement-associated responses at the malignant or other target tissue or . Macrophages are attracted into malignant breast tissue, but they remain inactivated, except for the production of tumor enhancing growth factors [Elgert et al. (1998) *J. Leukoc. Biol.* **64**:275]. T-cell response to the malignant tissue is inhibited, if not tolerized, to initiate apoptosis in the malignant tissue [Sotomayor et al. (1996) *Crit. Rev. Oncog.* 7:433; Bogen, B. (1996) *Eur. J. Immunol.* **26**:2671; Lauritzsen et al. (1998) *Int. J. Cancer* **78**:216]. The mechanism of tumor-induced tolerance has been the focus of numerous authors. The literature identifies numerous putative tumor-generated immune response suppressing products or mechanisms that allow a tumor cell to suppress one or another of the cellular components within an immune response. Many strategies are currently being tested that focus on specific immune responses, attempting to stimulate various portions of an immune response to effectively remove a tumor [Sogn, J.A. (1998) *Immunity* **9**:757]. Reported

treatments are relatively effective for newly injected experimental tumors. However, for treatment of older experimental tumors, "The probability of success drops rapidly, reaching essentially zero around 4 weeks after injection" [Sogn, J.A> (1998) supra]. Thus, 4 weeks appears to be the time required for injected tumor tissue, whether large or microscopic, to complete its control over a tumor suppressing immune response. In all probability the generally poor results obtained in clinical trials of effective experimental treatments are simply due to the maturity of malignant tissues before detection and treatment begins.

It has been recognized that the immune system can be stimulated to attack and remove long established malignant tissue [Coley, W.B. (1991) *Clin. Orthop.3*]. Results however, have not been consistent, and surgical protocols, with or without chemotherapy, have become the norm. This has especially been the case with breast cancer even though breast cancer was actually the first reported use of an immune stimulating agent in the treatment of cancer [Elgert et al. (1998) *supra*]. Since breast cancers are generally slow growing and tend to metastasize with time, the probability is high that malignant breast tissue has had more than ample time to tolerize any immune response, making the possibility of stimulating an adaptive immune response uncertain. However, Coley's and de Lisle's early work demonstrated that stimulating the immune system to remove a tumor, including inoperable breast cancer, is an inconsistent, but not an impossible task. It is likely that the extent of tissue specific tolerization will vary among patients, and therefore there is reason to believe that at least some patients can be cured with a properly stimulated adaptive immune response. How to cause this immune stimulation safely and efficaciously has been the focus of tumor immunology in recent years, but only with limited success.

There is a long-felt need in the art for improved and additional means for eliminating undesirable cells and tissues, e.g. treating cancer and infections, especially means which are not themselves harmful to the affected animal or human patient.

SUMMARY OF THE INVENTION

The present invention provides a method for mediating killing, inhibition and/or removal of a target cell, for example, a tumor cell or a virus- , pathogen- or protozoan-infected cell, or target tissue including, but not limited to malignant and benign tumors and neoplastic cells or tissue, from the body of a patient or an animal afflicted with a tumor or a

particular infected cell type, wherein the target cell or target tissue is characterized in that it exhibits at least one component on its cell surface which differs from a normal comparison cell. The present method comprises the steps of contacting target cells or target tissue with a random peptide or protein library (or a library bearing a ligand for at least one target cell or target tissue surface component bound to a carrier which is itself immunogenic) under conditions allowing specific binding of at least one member of the random peptide (or protein or other ligand) library, separating bound from unbound library members, isolating and amplifying the random peptide library members, immunizing an animal or human containing the target cell or target tissue, and allowing for the development of an immune response to the carrier for the random peptide (or ligand) library, and allowing for immune system-mediated destruction of the target cell or target tissue from the animal or human. The present invention also encompasses the use of a known ligand for a particular target cell, with the ligand being displayed on an immunogenic carrier, and being used in substantially the same way to direct attack on the target cell or target tissue, with the result that the target cell type is destroyed or the growth of the target cell or target tissue is inhibited, thus benefitting the animal or human to which the ligand-carrier is administered and to which there is an immunological response to the carrier. The use of a known ligand obviates the need for the use of a random peptide or protein display library and selecting (and optionally amplifying) members which specifically bind to the target cell or target tissue of interest. The animal or human can be immunized against the carrier prior to the procedures above, in which case it is not necessary to allow for the development of an immunological response to the carrier, or the immunological recognition of the carrier can be allowed to develop as a result of the one or more challenges with the random peptide or protein library or selected (and amplified) members of the library. In the case where a random peptide or protein library expressed in a filamentous bacteriophage is used, the animal or human can be subjected to administration of an immunogenic composition comprising the wildtype (wt) bacteriophage or the entire library. Specific binding of members from the random peptide (or ligand or protein) library in an animal or human which has immunological recognition of (antibody specific for or T cells specific for) the carrier then results in recognition of the target cell as foreign with antibody binding and complement-mediated destruction and/or recognition by cytotoxic T cells and destruction of the target cells or target tissue. Advantages of the present invention are that it

is not necessary to purify any one member of the random peptide or protein library and sequence the particular peptide region which is believed to mediate specific binding to the target cell or target tissue; the amplified members which bound to the target cell in the initial panning can be used after further rounds of selection and amplification or they can be used as is. Binding members of the population are easily selected. In the present method, the target cell can be a tumor cell, a virus-infected cell, a bacterium-infected cell or a protozoan-infected cell, among others or the target tissue can be neoplastic cells, a malignant tumor or a benign tumor or other neoplastic cells. Selection of members of the random peptide (or ligand) library which specifically bind to the target cell can be carried out *ex vivo* (such as using biopsy tissue from a tumor or using cultured tumor cells) or *in vivo*, for example, by injecting the library adjacent to the tumor or by intravenous injection. In one embodiment of the invention, library members specifically bound to the target cells or tissue can be separated by fluorescent activated cell sorting (FACS), for example after labelling of the target cells having the bound material with fluorescence-labeled antibody specific for a component of the carrier for the peptide or protein library. Then the selected phage or other selected library members can be recovered from their bound state and amplified. It is readily within the skill in the art to choose an appropriate diluent for the library and an appropriate route for contacting the library with target cells. In the case of target cells infected with a particular pathogen, one can do the selective binding *ex vivo* using cultured which are infected by the pathogen of interest, with differential binding studies to eliminate bound members of the population which recognize components of the normal cell or tissue.

The peptide (or protein or ligand) library can be incorporated as part of the surface of a bacteriophage, such as a filamentous phage of *Escherichia coli*, e.g., M13, f1 or fd, an other bacteriophage, or on the surface of a bacterial cell or as part of a protein or covalently bound to any immunogenic particle. The selected members (those which specifically bind with some affinity or avidity to the target cell or target tissue) from the library be capable of being separated from those members of the library which do not specifically bind to the target cell or target tissue, and it is desirable that the selected members can be amplified. Further, it is a crucial aspect of the present methods that the carrier on which the peptide expression library is expressed is itself recognized as immunogenic by the animal or human in which use is sought. Desirably the carrier elicits an anamnestic humoral response (antibody produced

specific for the carrier) and/or an cellular immune response. Further, it is desirable that the carrier itself and the carrier displaying the peptide or ligand library is not itself significantly harmful to the animal or human in which it is used.

Optionally, the administration of the selected (and desirably amplified) peptide or protein expressing phage can be via a composition which further comprises an agent which stimulates the immune response and/or which stimulates macrophages to attack the target tissue or target cells. Agent which stimulate the immune response can be, without limitation, interleukin-2 or Flt3. Agents which counteract the anti-inflammatory action of spermine include, but are not limited to, piperazine-like PI-Ca 91 and certain cytokines as known in the art.

This invention further includes the process of modifying and selecting groups of PPEB, that mimic, for antibody recognition and generation purposes, one or more epitopes of an invading pathogen, and the application of the selected group of PPEB as a colonizing mucosal vaccine which is incorporated into the normal flora of the mucosal lining or in bacteria that are added to the mucosal surface, and the products resulting therefrom. This invention also encompasses any peptide- or protein-expressing bacteria that are engineered with the use of bacteriophage selected for pathogen-mimicking epitopes from a PPEB library and the use of these modified bacteria and/or bacteriophage in a mucosal vaccine, with the proviso that the epitopes are produced on the mucosal over a period of time after the initial administration of the mucosal vaccine comprising the displayed peptide or protein. Desirably, there will be continued production of the antigen over a period of at least about two weeks. Administration of a persisting (and replicating) vaccine composition to a mucosal surface (nasal, oral, gastrointestinal, rectal or vaginal) results in the improved and longer lasting production of secretory antibodies (IgA) as well as circulating antibodies (primarily IgG and IgM).

BRIEF DESCRIPTION OF THE DRAWINGS

Figs. 1A-1B show exemplary schemes and methodologies of the present invention. Fig. 1A shows one possible selection scheme involving collection and amplification of tumor-associated PEPs. The subsequent step is incubation with tumor in suspension prior to Ficoll gradient separation and amplification of the PEPs associated with the cell pellet. This

process is then repeated with an amplification step between each selection component. Fig. 1B shows the treatment scheme used in a representative experiment, showing prior intraperitoneal (IP) vaccination with wt phage, subcutaneous injection of tumor at one or both sides of the back and sub-cutaneous treatment adjacent to the right side tumor on a 3 day interval.

Figs. 2A-2B graphically illustrate growth response over time comparing *in vitro* selected PEP treatment effects on EMT-6 mouse mammary tumors in the hairy litter mates of nude BALB/c mice. Untreated group includes mice without injections and mice receiving 1% BSA in PBS. The non-specific treatment group includes mice receiving treatments of wt phage, unselected library or PEPs selected on hen egg lysozyme. Treatment group includes mice receiving PEP treatments of selected Cys-6 body library or of the selected c7c tail library, 1 standard deviation bars are shown.

Fig. 3 shows the tumor growth response over time comparing *in vitro* selecting PEP treatment with the effects of untreated and non-specific treatments on EMT-6 tumors in normal BALB/c mice. These mice were purchased from a different source than the mice used in the experiment shown in Fig. 2. Unvaccinated groups consist of the same treatment protocol except that there was no prior wt phage vaccination given to these mice.

Fig. 4 shows a PEP selection series. This figure compares the plaque forming unit (PFU) in the cell pellet (closed bars) with the PFU of the immediately adjacent supernatant (open bars, after the incubation of the PEPs and EMT-6 and passage through a dilute Ficoll solution. Greater specificity of the selection process is shown by an increase in separation between the two bars at a given cycle of the panning process. Panning numbers 1-3 represent selection on EMT-6 through a Ficoll solution after the PEPs were isolated and amplified from tumor tissue. Pannings 4 and 5 show the reselection of panning #3 through additional rounds of selection on cultured EMT-6 and Ficoll separation.

Figs. 5A-5B show tumor mean size over time after *in vivo* selection of PEP treatment. Fig. 5A shows mean of all tumor size changes over time comparing *in vivo/in vitro* selected PEP treatment or wt phage treatment effects on EMT-6 tumors that were at a size of 200 mm or greater at the time of the first treatment. N number refers to the number of tumors at each point, standard error of the mean (SEM) bars are shown. Fig. 5B shows mean tumor size change for right side or left side tumors on wt treated or PEP treated mice.

Figs. 6A-6D illustrates immunohistochemical (red) staining for phage in tumor tissue of PEP treated and wt treated mice. Fig. 6A shows a left side tumor on a PEP treated mouse; Fig. 6B shows a right side tumor on the same PEP treated mouse as in Fig. 6A. The rightside tumor was adjacent to the site of treatment injection. Fig. 6C shows a left side tumor on a wt phage treated mouse. Fig. 6D shows a right side tumor on the same wt phage treated mouse as in Fig. 6C. This tumor was adjacent to the site of treatment injection.

DETAILED DESCRIPTION OF THE INVENTION

In the context of the present invention, a target cell or tumor tissue is one which is harmful (or otherwise undesirable) to an animal or human in which it occurs and/or is growing and dividing. A target cell can be a tumor cell or other neoplastic cell, a parasite-infected cell or a pathogen-infected cell or a newly fertilized egg. The pathogen or parasite can be viral, bacterial, fungal or protozoan. A target tissue can be a benign tumor, a malignant tumor or other neoplastic tissue. It is required by the present invention that the target cell have at least one component on its surface which distinguishes it from a comparable normal cell or tissue.

The random peptide expression library desirably includes all possible members of the selected length, or of the length which is necessary to mediate binding to a target cell or tissue. Generally, the number of consecutive amino acids needed to constitute a specific binding site is small, on the order of about 4 to about 12 amino acids. Where a longer peptide is displayed, the longer peptide region will encompass more than one theoretical binding site region and therefore the number of members in the inclusive library may be fewer than for the shorter peptides displayed. See, e.g. Brenner and Lerner (1992) *Proc. Natl. Acad. Sci. USA* 89:5381-5383 and other references cited hereinbelow for discussions of combinatorial chemistry and peptide display libraries. Also within the scope of the present invention are protein expression libraries, for example, those made by fragmenting the genome of a pathogen or parasite, wherein the protein of interest is expressed so that the protein is accessible for binding to the target cell or target tissue and is bound to a carrier which is immunogenic in the animal or human in which use is sought. It is also contemplated that the protein displayed on the carrier can be a ligand for the target cell or target tissue (but not for the comparable normal cells or normal tissue).

Random peptide or protein libraries for use in the present invention require that the peptides of random amino acid sequence are displayed in an accessible fashion so as to allow binding of particular peptide members to a target cell of interest. It is further required that the carrier for the expressed peptide is itself immunogenic. Thus, where a phage library is used, the peptide must be displayed on a surface protein of the phage. Filamentous bacteriophages suitable for random peptide library display include, without limitation, M13, f1, fd and pUSE5 [See, e.g., Parmley and Smith (1988) *Gene* 73:305-318; Devlin et al. (1990) *Science* 249:404-406; Civirla et al. (1990) *Proc. Natl. Acad. Sci. USA* 87:505-510; WO 91/18980; WO 92/06176; Bonnycastle et al. (1996) *J. Mol. Biol.* 258:747-762; United States Patent Nos. 5,427,908; 5,866,363; 5,723,286; 5,703,057; 5,432,018; 5,338,665; 5,270,170; among others; Kay et al. (1996) *Phage Display of Peptides and Proteins, A Laboratory Manual*, Academic Press, San Diego, CA). United States Patent No. 5,338,665 describes random peptide library display within the *lac* repressor protein. The use of the outer membrane protein *lamB* gene of *E. coli* for insertions has been described [see Charbit et al. (1988) *Gene* 70:181; Charbit et al. (1986) *EMBO J.* 5:3029; Charbit et al. (1987) *J. Immunol.* 139:1658].

It has been shown that tumor cells and most infected cells generally express different markers or proteins on their surface than do normal or uninfected of the same type [Mariane et al. (1995) *Cancer Research Suppl.* 55:5911s-5915s]. Alternatively, the target cell or tissue can express the same surface components as normal tissue but in such greatly increased numbers that panning with an expression/display library results in selection for particular binding members. Since peptide or protein expressing bacteriophage (PEP) libraries (for the purpose of this patent, the term PEP encompasses any carrier having at least one immunogenic component and with a peptide or protein that can be selected for and replicated or chemically synthesized) have peptides and proteins which have been shown to adhere to many cell types and epitopes [Bonnycastle et al. (1996) J. Molec. Biol. **258**:247-762; Smith and Scott (1993) *Meth. Enzymol.* **217**:228-257], in theory at least one library member can be selected for specific or preferential binding to a particular target cell or target tissue. Alternatively, a ligand known to bind to a particular cell or tissue can be incorporated in or linked to the immunogenic carrier.

Others have shown that an immune response can be directed against some types of tumors by treatments such as killed or attenuated bacterial injections, anti-tumor antibody

injection or bivalent Fab fragments specific for the tumor cell. Although sometimes effective, these treatments have not proven to be generally effective in the long term or with vigorously growing tumors. A novel scheme for directing an immune response against tumor (implanted in BALB/c mice as exemplified) is presented herein. The scheme is based upon the knowledge that (1) Peptide Expression Phage (PEP) can be selected from PEP libraries that will bind to specific antigens or (2) specific PEPs can be identified that will localize to specific tissue within mice or (3) wild type (wt) Phage as well as PEP library phage are strongly immunogenic or (4) other display systems can be used. Other display systems include bacterial, yeast or mammalian cells. PEPs can be selected that specifically bind to tumor cells, tumor tissue or other target cells or tissue, and that PEP localization to tumor tissue directs immune responses against the selected PEP/tumor complex (Fig. 1A).

To determine the validity of this hypothesis, PEPs were selected for use in the treatment, using conventional panning techniques on the EMT-6 tumor cell line in culture. Figs. 2 and 3 represent the results of treatment obtained by this method of "panning". A reduction of the tumor rate of growth was identified, but not a complete removal of the tumors. Fig. 1C outlines the treatment protocol used in these experiments.

Fig. 2 represents the mean rate of growth of tumors on mice treated in three different groups. This experiment was to determine if the treatment effect was specific to the selected PEPs. The treatment protocol shown in Fig. 1C was followed except that tumors were only injected on the right side of the mice. Only the target cell- or target tissue-selected PEP treatment was sufficient to reduce the tumor growth over time. This is shown by the separation of the untreated and PEP treatment standard deviation error bars after five treatments ($p=0.03$). The significance of this was that only the PEP treated group was successful in reducing the tumor growth rate.

The experiment depicted in Fig. 3 used the same EMT-6 and phage treatment solutions as Fig. 2, but treatment solutions were supplied in coded vials to make this a blind study. This experiment confirmed reduction rates of tumor growth obtained in the experiment illustrated in Fig. 2 in a completely different mouse colony, and clearly shows that prior vaccination is required for PEP treatment to be effective ($p=0.005$).

With the exception of mice used in the experiment shown as Fig. 3, all mice used in these experiments were hairy littermates of a BALB/c nude mouse colony maintained at the

University of Nevada. In these hairy littermate mice, the EMT-6 cell line has only been effective in establishing vigorous tumors in 50% of the tumor injections. During the first two weeks after injection, it was not clear whether any tumor under 150 mm^3 would grow or spontaneously regress. Where the tumor cells are allogeneic to the mouse into which they are injected to initiate tumor formation, tumors are not always established in a stable fashion. Where the injected tumor cells are isogeneic to the mouse into which they are injected, there is essentially no spontaneous tumor regression. Once tumor mass reached a size of 150 mm^3, it grew vigorously until it overcame the animal. The mice used to generate the data in Fig. 3 were BALB/c mice purchased from Harlan Laboratories. These syngeneic mice did not demonstrate spontaneous regression of the injected tumors. Because of the availability of two different types of libraries (Fig. 1B), a 6 amino acid (aa) cysteine constrained pVIII library and a 7 aa cysteine constrained pIII library, we tested each library individually. In the experiment shown in Fig. 2 there was no significant difference between these two libraries. In the experiment shown in Fig. 3, the tail library PEPs demonstrated some improvement in reduction of growth rates of tumors on non-treated sides of mice vs. the body library PEPs. Taken together, the difference was not considered sufficiently significant to use one library over the other and pooled individually selected libraries in future treatments.

To attempt to improve the efficacy of PEP treatment, the PEP selection scheme shown in Fig. 1D was developed. In this scheme the complete PEP library was first injected into tumor-bearing mice, then after three hours a biopsy of tumor tissue was removed, and the PEPs within the tumor tissue were amplified. The amplified PEPs were then incubated with tumor in suspension. The tumor and any associated PEPs were passed through a dilute Ficoll solution at low speed centrifugation to remove the cell/PEP complexes from the background phage, which generally remained in solution. Fig. 4 shows results of this panning protocol using selection through three tumor mice with one round of associated cell selection each (pannings 1-3). Two additional rounds of cell selection were also performed on the PEPs from the third tumor selection (pannings 4 and 5). After the Ficoll centrifugation step, we determined the relative selectivity of the panning round by comparing the difference in the number of PEPs associated with the cell pellet to the number of phage in the immediately adjacent supernatant. Fig. 4 shows the improvement after three rounds of tumor selection, but

illustrates only minimal additional selection during further cell associated separation steps (pannings 4 and 5).

Fig. 5A shows the results of tumor treatment with the PEPs obtained from panning 4 ($p=0.01$), as identified in Fig. 4. Here, for the first time, tumor regression to undetectable size in some of the mice was observed. All treatments were given subcutaneously adjacent to right side tumors. The data in Fig. 5B compares right flank tumor and left flank tumor for wt vs. PEP treated mice. In PEP treated mice, the rate of decrease in mean tumor size for right flank tumors is changing faster than the rate of decrease for left flank tumors. In the wt phage treated mice, however, the growth rate of right side vs. left side tumors does not change. Note the overlap in the standard error of the mean (SEM) error bars at the three week time point, which negates the apparent change in slope of the growth of left flank tumor vs. right flank tumor at three weeks. The difference in the rate of decrease between right flank and left flank tumors in PEP treated mice can be explained because treatments were injected at the right flank. The PEPs should be reduced in number by the time they circulate through the body to arrive at the left flank tumor. Therefore less treatment effect was predicted in the left flank tumor than in the tumor adjacent to the site of injection. The wt phage treatment does not result in significant differences in the growth rate of right side tumors vs. left side tumors, as would be expected for a phage that did not preferentially localize to any particular tissue.

Figs. 6A and 6B show immunohistochemical staining for the presence of phage within the tumor tissue. Comparison of right side and left side tumor staining confirm the prediction that there is a substantially stronger phage presence at right side (treated) tumor than at left side (untreated) tumor. Note the lack of signal in the wt phage treated tumors on either right side or left side (Figs. 6C and 6D). This result supports the hypothesis that the selected PEPs are localizing to the tumor tissue and causing an immune response to remove tumors. In addition, wt phage do not localize at the tumor and thus do not cause removal of the tumor tissue.

In certain experiments histological examination of the tumors in the mice immunized with the wt phage and treated with the selected PEP phage revealed that the tumors showed a massive infiltration of neutrophils and macrophages. The tumors in these mice also exhibited localization of the selected PEP phage in the tumor, and some internalization of phage in the macrophages and neutrophils. No localization of wt phage was observed in the nonspecific

treatment group which was injected with a PEP phage preparation which had not been selected on the tumor tissue or tumor cells.

Similar results have been obtained in C57Bl/6 mice pre-immunized with the wt phage carrier and then into which tumor-producing melanoma (B16) and sarcoma (MCA-205) were injected. After tumors developed, specifically binding peptide-expressing phage were selected and amplified and injected into the cognate tumor-bearing mice. In at least one experiment, many mice (with EMT-6 tumors) injected with the tumor-selected phage exhibited signs of tumor necrosis as early as 18-24 hours after challenge with the injected phage. Without wishing to be bound by theory, it is believed that the sarcoma and melanoma tumors had reached too large a size before treatment too allow for such dramatic results.

PEPs from a short (6 random amino acid insert) peptide PEP library can be selected to localize to tumor tissue and the usage of these selected PEPs will cause an immune response that will reduce the growth of tumor tissue. The specificity of the selection protocol determines the extent of tumor removal. This novel method of redirecting the immune system toward removal of tumor provides a new tool to ameliorate immune response to tumors.

The present invention relies on production of an immune response to the carrier (or some component of the carrier) for the target cell-binding peptide (or protein or other ligand). The immune response can be humoral, i.e., the production of polyclonal and/or monoclonal antibodies capable of specifically binding to at least one component or epitope of the carrier, desirably the wild-type carrier, wherein that epitope is not disrupted by the insertion of the peptide-encoding portion in the construction of the random library nor is access of the antibody to the carrier immunogenic component blocked by the expressed peptide or protein. The term antibody is used to refer both to a homogenous molecular entity, or a mixture such as a serum product made up of a plurality of different molecular entities. Monoclonal or polyclonal antibodies specifically reacting with the carrier can be made by methods known in the art. See, e.g., Harlow and Lane (1988) *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratories; Goding (1986) *Monoclonal Antibodies: Principles and Practice*, 2d ed., Academic Press, New York; and Ausubel et al. (1987)Current Protocols in Molecular Biology, Wiley Interscience. Also, recombinant immunoglobulins may be produced by methods known in the art, including but not limited to, the methods described in U.S. Patent

No. 4,816,567. Monoclonal antibodies with affinities of 10^8 M^{-1}, preferably 10^9 to 10^{10} or more are preferred. Alternatively, the immune response specific to and directed against the carrier can be a cell-mediated immune response wherein, that epitope is not destroyed or disrupted by the insertion of the peptide coding sequence during the construction of the random peptide library.

Antibodies specific for the carrier for the peptide, protein or ligand which specifically binds to the target cell are useful, for example, for detecting the presence of target cell surface structures bound to a selected binding partner in a test sample. Additionally, these antibodies play a key role in marking the target cell for destruction by the immune system and by non-specific defense mechanisms. Frequently, the antibodies are labeled by joining, either covalently or noncovalently, a substance which provides a detectable signal. Suitable labels include but are not limited to radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent agents, chemiluminescent agents, magnetic particles and the like. United States Patents describing the use of such labels include, but are not limited to, Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241.

Compositions and immunogenic preparations, including vaccine compositions, comprising substantially purified carrier or an immunogenic peptide of the carrier capable of inducing protective immunity in a suitably treated mammal and a suitable excipient therefor are provided. Alternatively, hydrophilic regions of the a surface component of the carrier, for example, hydrophilic regions of a protein component(s) of the carrier can be identified by the skilled artisan, and antigenic carrier-derived peptides can be synthesized and conjugated to a suitable protein such as bovine serum albumin or keyhole limpet hemocyanin) if needed for use in vaccines or in raising antibody specific for the carrier of the present intention. Immunogenic compositions are those which result in specific antibody production when injected into a human or an animal. Such immunogenic compositions or vaccines are useful, for example, in immunizing an animal, including humans, against the carrier for the random peptide library from which target cell binding members are isolated and amplified. The vaccine preparations comprise an immunogenic amount of a wild type carrier or an immunogenic fragment(s) or subunit(s) thereof. Such vaccines can comprise one or more carrier antigens or in combination with another protein or other immunogen, or an epitopic peptide derived therefrom. An "immunogenic amount" means an amount capable of eliciting

the production of antibodies directed against the carrier which is to be used in isolation of the target cell binding peptide and subsequent treatment in an individual or animal to whom or which the vaccine has been administered.

Immunogenic adjuvants and/or excipients can be used to enhance the immunogenicity of the carrier proteins or other antigenic components of the carrier, or peptides derived in sequence from the foregoing. Such added components of an immunological composition can include, without limitation, proteins and polysaccharides, liposomes, and bacterial and membranes. Protein carriers may be joined to the proteins or peptides derived from the carrier to form fusion proteins by recombinant or synthetic means or by chemical coupling. Useful carriers and means of coupling such carriers to polypeptide antigens are known in the art.

The immunogenic compositions may be formulated by any of the means known in the art. They are typically prepared as injectables, either as liquid solutions or suspensions. Solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared. The preparation may also, for example, be emulsified, or the carrier, protein(s)/peptide(s) encapsulated in liposomes. Where mucosal immunity is desired, the immunogenic compositions advantageously contain an adjuvant such as the nontoxic cholera toxin B subunit (see, e.g., United States Patent No. 5,462,734). Cholera toxin B subunit is commercially available, for example, from Sigma Chemical Company, St. Louis, MO. Other suitable adjuvants are available and may be substituted therefor. It is preferred that an adjuvant for an aerosol immunogenic (or vaccine) formulation is able to bind to epithelial and stimulate mucosal immunity.

Among the adjuvants suitable for mucosal administration and for stimulating mucosal immunity are organometallopolymers including linear, branched or cross-linked silicones which are bonded at the ends or along the length of the polymers to the particle or its core. Such polysiloxanes can vary in molecular weight from about 400 up to about 1,000,000 daltons; the preferred length range is from about 700 to about 60,000 daltons. Suitable functionalized silicones include (trialkoxysilyl) alkyl-terminated polydialkylsiloxanes and trialkoxysilyl-terminated polydialkylsiloxanes, or example, 3-(triethyoxysilyl) propyl-terminated polydimethylsiloxane. See United States Patent No. 5,571,531, incorporated by reference herein. Phosphazene polyelectrolytes can also be incorporated into immunogenic

compositions for transmucosal administration (intranasal, vaginal, rectal, respiratory system by aerosol administration) (See United States Patent No. 5,562,909).

The selected (and desirably amplified) peptide- or protein-expressing phage or other peptide or protein-carrier complexes are administered to a human or animal for whom elimination of a target cell type or target tissue is desired within a formulation comprising an excipient or diluent which is appropriate for *in vivo* use and suitable for the chosen route of administration. Optionally, the administered therapeutic composition further comprises one or more agents in amounts effective to stimulate the immune response of the human or animal and/or to stimulate mononuclear cells to attack the target cells or tissue. Agents which stimulate the immune response include, without limitation, interleukin-2 [Chang and Shu (1996) Crit. Rev. Oncol. Hematol. **22**:213; Nelson and Nelson (1988) Immunol. Cell Biol. **66**:97] and Flt [Lynch et al. (1997) Nat. Med. **3**:97; Pulendran et al. (1998) J. Exp. Med. **188**:2075]. Spermine blockers have been shown to stimulate tumor elimination or inhibition in treatment regimens other than those disclosed herein; it is believed that the spermine blocker piperazine-like PI-Ca 91 counters the anti-inflammatory action of spermine and thus, allows for the activation of tumor resident macrophages [Zhang et al. (1997) J. Exp.. Med. **185**:1759]. Thus, any cytokine or other agent which is known in the art as stimulating or enhancing the inflammatory is useful in the selected peptide- or protein-carrier containing compositions of the present invention. It is desired that the peptide- or protein-carrier be present in the compositions for treatment in an amount effective for stimulating the immune system and other defense mechanisms in the human or animal bearing target cells or target tissue to be eliminate or to be inhibited in growth. It is contemplated that the number of particles of the selected agent be from about 10^7 to about 10e14 per dose. Repeated doses may be necessary at intervals of from about 3 to about 30 days are contemplated to effectively remove or inhibit growth of target cells or tissue, especially in the case of tumor tissue. It is desired that there be administration of immunogenic compositions comprising the carrier or carrier antigens prior to treatment with selected material.

The active immunogenic ingredients are often mixed with excipients or carriers which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients include but are not limited to water, saline, dextrose, glycerol, ethanol, or the like

and combinations thereof. The concentration of the immunogenic carrier or carrier polypeptide in injectable formulations is usually in the range of 0.2 to 5 mg/ml.

In addition, if desired, the immunological compositions may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, and/or adjuvants which enhance the effectiveness of the vaccine. Examples of adjuvants which are effective include, but are not limited to, aluminum hydroxide; N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP); N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine (CGP 11637, referred to as nor-MDP); –acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3hydroxyphosphoryloxy)-ethylamine (CGP 19835A, referred to as MTP-PE); and RIBI, which contains three components extracted from bacteria, monophosphoryl lipid A, trehalose dimycolate and cell wall skeleton (MPL+TDM+CWS) in a 2% squalene/Tween 80 emulsion. The effectiveness of an adjuvant may be determined by measuring the amount of antibodies directed against the immunogen resulting from administration of the immunogen in vaccines which are also comprised of the various adjuvants. Such additional formulations and modes of administration as are known in the art may also be used.

Carrier, carrier protein and/or epitopic fragments or peptides of sequences derived therefrom can be formulated into immunological compositions as neutral or salt forms. Pharmaceutically acceptable salts include, but are not limited to, the acid addition salts (formed with free amino groups of the peptide) which are formed with inorganic acids, e.g., hydrochloric acid or phosphoric acids; and organic acids, e.g., acetic, oxalic, tartaric, or maleic acid. Salts formed with the free carboxyl groups may also be derived from inorganic bases, e.g., sodium, potassium, ammonium, calcium, or ferric hydroxides, and organic bases, e.g., isopropylamine, trimethylamine, 2-ethylamino-ethanol, histidine, and procaine.

The carrier-specific immunogenic compositions or vaccines are administered in a manner compatible with the dosage formulation, and in such amount as prophylactically and/or therapeutically effective. The quantity to be administered, generally in the range of about 1 to 5,000 μg of carrier or protein per dose, more generally in the range of about 50 to 500 μg of protein per dose, depends on the subject to be treated, the capacity of the individual's immune system to synthesize antibodies, and the degree of protection desired. Precise amounts of the immunogen may depend on the judgment of the physician or

practitioner and may be peculiar to each individual, but such a determination is within the skill of such a practitioner.

The vaccine or other immunogenic composition can be given in a single dose or multiple dose schedule. A multiple dose schedule is one in which a primary course of vaccination may include 1 to 10 or more separate doses, followed by other doses administered at subsequent time intervals as required to maintain and or reinforce the immune response, e.g., at 1 to 16 weeks for a second dose, and if needed, a subsequent dose(s) after several months.

The selection of the group of PPEB from a PPEB library by antisera from a host immune system to the pathogen in question allows the production of an "epitope" vaccine. This selection for other than HIV-1 pathogens has been established for filamentous bacteriophage [See, e.g., Folgori et al. (1994) *EMBO J.* 13:2236-2243]. It is, however, the use of these selected PPEB as a colonizing mucosal vaccine rather than a humoral systemic vaccine that is unique to our process. The PPEB that mimic the pathogen's epitopes are then used as the immunizing agent. These PPEB must then be able to colonize the mucosal surface which is the point of entry of the pathogen, at least for a time reasonable enough to establish an immune response and for the reapplication of the vaccine as necessary to provide sufficient stimulation of the immune response.

This system of mucosal stimulation is practical with any bacteriophage that can be made to express a peptide or protein on one or more of its surface proteins, and that can be selected on the basis of its avidity to antisera produced by the pathogen in question, and that can induce an immune response to provide some level of protection from infection. This system will also be practical with any bacteriophage that can cause a host bacteria, whether resident or added to the mucosal surface in question, to express a selectable peptide or protein that can then be recognized by the immune system and cause a mucosal immune response.

An exemplary scheme for the selection of the PPEB group that will generate an immune response that mimics the immune response created by the invading pathogen is as follows. Surfaces referred to in this scheme can be essentially any solid support: plate surfaces, bead surfaces, the surfaces within any type of appropriate column, or even the surface of a lysosome or a cell that expresses the protein or peptide on its surface.

1) Infect appropriate mammal with pathogen.

2) Incubate with complete PPEB library.

3) Remove supernate and incubate on plate with bound anti-sera. Note that it is important to provide sufficient incubation with normal sera to remove all members of the PPEB library that bind to normal sera.

4) Remove bound PPEB only and amplify.

5) Multiple rounds of "panning" to select PPEB that only bind to anti-sera.

6) Multiple rounds of "panning" to select PPEB that only bind to antibody.

7) Inject selected PPEB into mammal to produce anti-sera (#2) to the selected PPEB.

8) Incubate anti-sera #2 with the pathogen.

9) Assay to identify selected PPEB that will produce neutralizing anti-sera.

10) Vaccinate mammal as appropriate with selected PPEB to produce protective anti-sera and then infect mammal with pathogen to determine degree of protection produced by the selected PPEBs used as a vaccine.

Mucosal vaccines of the present invention colonize and/or replicate on the mucosal surfaces to which they are administered and present antigen in a substantially continuous fashion, desirably for a period of at least about two weeks. Mucosal vaccines and immunogenic compositions can be administered via routes including intranasal, oral, inhaled, intragastric, vaginal, and anal (rectal) routes. Bacteria expressing an antigen of interest colonize the mucosal surface, continually presenting antigen. Recombinant viruses expressing either selected epitopes or (larger) known antigens of the pathogen of interest infect and are replicated by resident microbial flora on the mucosal surface or are replicated by mucosal cells. Where known antigens are to be inserted, cloning is carried out using techniques well understood by and readily accessible to those of ordinary skill in the art of molecular biology.

Note that for the example of an HIV-1 vaccine, all multiple inoculation routes include a vaginal application component for females. This can be substituted as an anal component for males. A feature of a PPEB delivery system is that PPEB can be administered monthly to the female via products generally in use to guard against blood leakage (especially via tampons) during the menstruation cycle, although vaccine testing in small animals is anticipated to be accomplished using liquid suspension of the PPEBs and inoculation. The

application of the vaccine during menstruation also coincides with the time in the menstruation cycle when the female vaginal immune system is most responsive to activation. Additionally, a colonized female can transfer colonization to a male during intercourse, as has been demonstrated in prior art references. Because example #1 used herein is an HIV-1 vaccine, this figure focuses on the human female, who is at greater risk from acquiring HIV-1 infection through intercourse than a male.

Except as noted hereafter, standard techniques for peptide synthesis, cloning, DNA isolation, amplification and purification, for enzymatic reactions involving DNA ligase, DNA polymerase, restriction endonucleases and the like, and various separation techniques are those known and commonly employed by those skilled in the art. A number of standard techniques are described in Ausubel et al. (1994) *Current Protocols in Molecular Biology*, Green Publishing, Inc., Sambrook et al. (1989) *Molecular Cloning, Second Edition*, Cold Spring Harbor Laboratory, Plainview, New York; Maniatis et al. (1982) *Molecular Cloning*, Cold Spring Harbor Laboratory, Plainview, New York; Wu (ed.) (1993) Meth. Enzymol. **218**, Part I; Wu (ed.) (1979) Meth Enzymol. **68**; Wu et al. (eds.) (1983) *Meth. Enzymol.* **100** and **101**; Grossman and Moldave (eds.) *Meth. Enzymol.* **65**; Miller (ed.) (1972) Experiments in *Molecular Genetics*, Cold spring Harbor Laboratory, Cold Spring Harbor, New York, Old Primrose (1981) *Principles of Gene Manipulation*, University of California Press, Berkeley; Schleif and Wensink (1982) *Practical Methods in Molecular Biology*; Glover (ed.) (1985) *DNA Cloning* Vol. I and II, IRL Press, Oxford, UK; Hames and Higgins (eds.) (1985) *Nucleic Acid Hybridization*, IRL Press, Oxford, UK; Setlow and Hollaender (1979) *Genetic Engineering: Principles and Methods*, Vols. 1-4, Plenum Press, New York. Abbreviations and nomenclature, where employed, are deemed standard in the field and commonly used in professional journals such as those cited herein. All patents, patent publications and other references cited in this application are incorporated by reference in their entirety to the extent that they are not inconsistent with the present Specification.

The foregoing discussion and the following examples illustrate but are not intended to limit the invention. The skilled artisan will understand that alternative methods can be used to implement the invention.

EXAMPLES

Example 1 Materials and Experimental Animals

Mice used as experimental animals were either the hairy littermates of a nude +/- BALB/c colony maintained at the University of Nevada, Reno, or BALB/c mice (Harlan Laboratories (Indianapolis, IN). The mice were 8 to 15 weeks old at the start of the experiments described herein.

Wildtype (wt) filamentous bacteriophage fd was obtained from the American Type Culture Collection (Manassas, VA). A random peptide-expressing bacteriophage library (Cys-6) was obtained from George Smith (University of Missouri, Columbia, MO) (Scott and Smith (1990) Science **249**:386-390). Random peptide display libraries are also commercially available: filamentous bacteriophage, New England Biolabs, Beverly, MA; in an *E. coli* flagellin-thioredoxin fusion protein, Invitrogen, Carlsbad, CA; in a T7 protein, Novagen, Madison, WI; for example. Other peptide library display systems are also available from Amersham, Pharmacia Biotec, Syncomm Corporation, and Display Systems Biotech. Random peptide display vectors and methods for manipulating them are described in numerous readily accessible sources; see, for example, United States Patents 5,223,409; 5,403,484; 5,579,898; 5,338 665 (within *E. coli* lac repressor protein); see also Kay et al. (1996) *Phage Display of Peptides and Proteins, A Laboratory Manual*, Academic Press, San Diego, CA, and references cited therein.

Example 2: Phage Expansion and Assays.

Phage were propagated in 2xYT medium inoculated with uninfected *E. coli* (XLI-Blue MRF' from Stratagene, San Diego, CA) grown with vigorous aeration overnight at 37°C. Phage were obtained after centrifugation to remove the bacteria and then incubation of the supernatant in polyethylene glycol, average molecular weight 8000 (PEG-8000) (37mM) and NaCl (0.5M) for 30 min. at 4°C. The phage were centrifuged out of the solution at 10,000 x g for 20 min. The PEP-containing supernatant was removed by draining for 30 min., and the phage-containing pellet was resuspended in 1% bovine serum albumin (BSA) in PBS. The phage-containing solution was heat inactivated at 65°C for 10 min., and then the debris was centrifuged out of the solution at 14,000 x g. The supernatant containing the phage was then titered in a soft agar overlay containing XL1-Blue MRF' bacteria on a LB agar plate.

Typically at $1x10^{12}$ PFU per ml of solution were obtained for each 10 ml of medium in the overnight growth step.

Example 3: PEP Selection and Treatment.

In vivo selection began with the intraperitoneal (IP) or intravenous (IV) injection of $1x10^{11}$ library phage into a tumor-bearing mouse. After 3 hours the mouse was sacrificed, and a section of the tumor mass was removed aseptically. This was mixed with a fresh culture of phage-free, male *E. coli* and amplified overnight with or without antibiotic selection, depending upon the library used. The phage were extracted per above and titered. $1x10^{7}$ or $1x10^{9}$ phage were then incubated with $1x10^{6}$ in suspension overnight at 4°C or for 60 min. at room temperature. The cell pellet with attached phage was resuspended in 1 ml of medium and centrifuged over a 3:1 mixture of cold Histopaque-1077:PBS at low speed(95 x g for 20 min.) so that unbound phage are not also pelleted. (Histopaque-1077 is a trademark of Sigma Chemical Co., St. Louis, MO; it is Ficoll solution having a density of 1.077 g/ml, nonionic, synthetic polymer of sucrose, average molecular weight 400,000 d. The cell pellet was resuspended in 1 ml medium and the wash process repeated. The PEPs in the pellet were then amplified as described above. The entire process was repeated three times. Then $1x10^{11}$ PEPs in 100 µl of 1%BSA in PBS was used for each treatment injection. Treatment injections were subcutaneous adjacent to, but not into, the right side tumor.

Example 4: Tumor Injection, Treatment and Measurements.

The EMT-6 mammary tumor cell line [Rockwell, S. (1972) J. Natl. Cancer Institute **49**:735-749] is routinely grown under 5% CO_2, 95% humidity at 37°C in Iscove's Modified Dulbecco Medium supplemented with 10% fetal calf serum, penicillin, and streptomycin. were extracted with 0.2% trypsin, twice washed in medium, counted and resusupended at appropriate concentrations in Hanks Balanced Salt Solution (or in fresh culture medium). 100 µl of the tumor cell suspension (typically 1×10^{6} to 3×10^{6}) was injected subcutaneously at the right and left flanks (sides of the mid-back region) of each mouse. Tumors were allowed to develop for 7-10 days before challenge. Measurements of tumors was immediately prior to treatment and at intervals as indicated in the discussion hereinabove. Tumors were measured using caliper readings of tumor length, width and thickness Tumor

volume (size) was calculated as (length x width x thickness)/2 as mm^3 or as the sum of length plus width/2 for average diameter measurements. Desirably in the animal models experiments, the injected tumor are syngeneic with the experimental animal.

Example 5: Mucosal Vaccine for HIV-1 Protection.

The primary route of HIV-1 infection is thought to be through the mucosal surfaces, primarily anal or vaginal. Using known methods [interalia, Folgor, et al. (1994) supra; Ausubel et al. (1995) supra; Smith and Scott (1993) *Meth. Enzymol.* 217:228-257] a PPEB library can be generated. The library can be made of some number of amino acids (about 4 to at least about 20) as a peptide insert/s within the pVIII protein of filamentous bacteriophage or peptide or protein inserts into the pIII protein of filamentous bacteriophage. The PPEB can be selected using normal sera in combination with antisera to eliminate PPEBs that bind to normal sera, and then amplifying the PPEBs that bind to the antisera. The PPEB thus selected are isolated, amplified and used to generate antibodies in a small animal model, such as mice. The generated antibodies are then tested *in vitro* for avidity and neutralizing capability to the HIV-1 strain used to generate the antisera. The PPEB(s) that generate the highest avidity and the most neutralizing antibodies to HIV-1 can then be pooled and used as a mucosal vaccine.

WHAT IS CLAIMED IS:

1. A method for inhibiting growth of a target cell or target tissue, said target cell or target tissue being distinguished from normal cells or normal tissue in at least one surface component in presence or amount, said method comprising the steps of:

(a) contacting a target cell or target tissue with a peptide or protein expression library, said peptide expression library displaying expressed peptides covalently linked to a carrier;

(b) recovering at least one selected member of the peptide or protein expression library bound to the target cell or target tissue in step (a);

(c) administering to a human or animal having a target cell or target tissue to be inhibited in its growth a composition comprising at least one member of the selected peptide expression library recovered in step (b);

(d) allowing the human or animal to which the composition comprising at least one selected member of the peptide or protein expression library recovered in step (b) to develop an immunological response to said composition,

whereby growth of the target cell or target tissue is inhibited due to immunological response of the animal or human to the carrier for the peptide or protein expression library.

2. The method of claim 1 further comprising the step of amplifying the at least one member of the peptide or protein expression library which had been recovered from the target cell or target tissue prior to the step of administration to the animal or human.

3. The method of claim 1 wherein the target cell is a tumor cell.

4. The method of claim 1 wherein the target cell is a cell infected with a virus, a bacterium, a fungus or a protozoa.

5. The method of claim 1 wherein the target cell is a neoplastic cell.

6. The method of claim 1 wherein the target cell is a newly fertilized egg.

7. The method of claim 1 wherein the target cell is tumor tissue.

8. The method of claim 1 further comprising the step of administering to the animal or human having a target cell or target tissue to be inhibited in its growth an immunogenic composition comprising the carrier on which the peptide or protein is displayed prior to step (c).

9. The method of claim 1 wherein the carrier for the peptide or protein expression library is a virus.

10. The method of claim 9 wherein said carrier is a bacterial virus.

11. The method of claim 10 wherein said carrier is a filamentous bacteriophage.

12. The method of claim 11 wherein said filamentous bacteriophage is f1, fd or M13.

13. The method of claim 1 wherein the carrier for the peptide or protein expression library is a protein which is immunogenic in the animal or human having a target cell or target tissue for which growth inhibition is desired.

14. The method of claim 1 wherein the carrier is a carbohydrate or aggregated protein.

15. The method of claim 1 wherein the carrier is a prokaryotic cell or a eukaryotic cell.

16. The method of claim 1 wherein the composition comprising the at least one selected peptide or protein expression library further comprises an agent in an amount effective for stimulating an immune response of the human or animal in which it is administered or agent in an amount effective for enhancing an inflammatory response of the human or animal in which it is administered.

17. The method of claim 16 wherein the composition comprises an agent selected from the group consisting of a cytokine, interleukin-2, Flt3 or PI-Ca 91.

18. The method of claim 1 wherein the target cell or target tissue is distinguished from a normal cell or normal tissue by the presence of at least one component on the surface of the target cell or target tissue.

19. The method of claim 1 wherein the target cell or target tissue is distinguished from a normal cell or normal tissue by the presence of more copies of at least one surface component on the target cell or target tissue than is present on the comparison normal cell or normal tissue.

20. The method of claim 1 wherein inhibition of growth of the target cell or target tissue is mediated by opsonization of a complex comprising a target cell or target tissue bound by at least one member of the peptide or protein expression library, wherein an antibody specific to an immunogenic component of the carrier of the peptide expression library has bound to said complex.

21. A method for inhibiting growth of a target cell or target tissue in a n animal or human in which inhibition of said target cell or target tissue is desired, said target cell or target tissue being distinguished from normal cells or normal tissue in at least one surface component in presence or amount, said method comprising the steps of:

(a) contacting a target cell or target tissue with a ligand which specifically binds to the target cell or target tissue and which substantially does not bind to normal

cells or normal tissue, wherein said ligand is diplayed on the surface of and covalently linked to a carrier which is immunogenic in the animal or human into the ligand displayed on the surface of a carrier is administered, expressed peptides covalently linked to a carrier;

(b) allowing the human or animal to which a composition comprising the ligand displayed on the surface of the carrier to develop an immunological response to said composition,

whereby growth of the target cell or target tissue is inhibited due to immunological response of the animal or human to the carrier for the ligand.

22. The method of claim 21 wherein the target cell is a tumor cell, a cell infected with a virus, a bacterium, a fungus or a protozoa, a neoplastic cell or a newly fertilized egg.

23. The method of claim 21 wherein the target cell is tumor tissue.

24. The method of claim 21 further comprising the step of administering to the animal or human having a target cell or target tissue to be inhibited in its growth an immunogenic composition comprising the carrier on which the ligand is displayed prior to administration of the composition comprising the ligand which specifically binds to the target cell or target tissue..

25. The method of claim 21 wherein the carrier for the peptide or protein expression library is a bacterial virus, a mammalian virus, a prokaryotic cell, a yeast cell, mammalian cell, a protein, carbohydrate or aggregated protein.

26. The method of claim 25 wherein said filamentous bacteriophage is f1, fd or M13.

27. The method of claim 21 wherein inhibition of growth of the target cell or target tissue is mediated by opsonization of a complex comprising a target cell or target tissue

bound by at least one member of the peptide or protein expression library, wherein an antibody specific to an immunogenic component of the carrier of the peptide expression library has bound to said complex.

28. The method of claim 21 wherein the composition comprising the at least one selected peptide or protein expression library further comprises an agent in an amount effective for stimulating an immune response of the human or animal in which it is administered or agent in an amount effective for enhancing an inflammatory response of the human or animal in which it is administered.

29. The method of claim 28 wherein the composition comprises an agent selected from the group consisting of a cytokine, interleukin-2, Flt3 or PI-Ca 91.

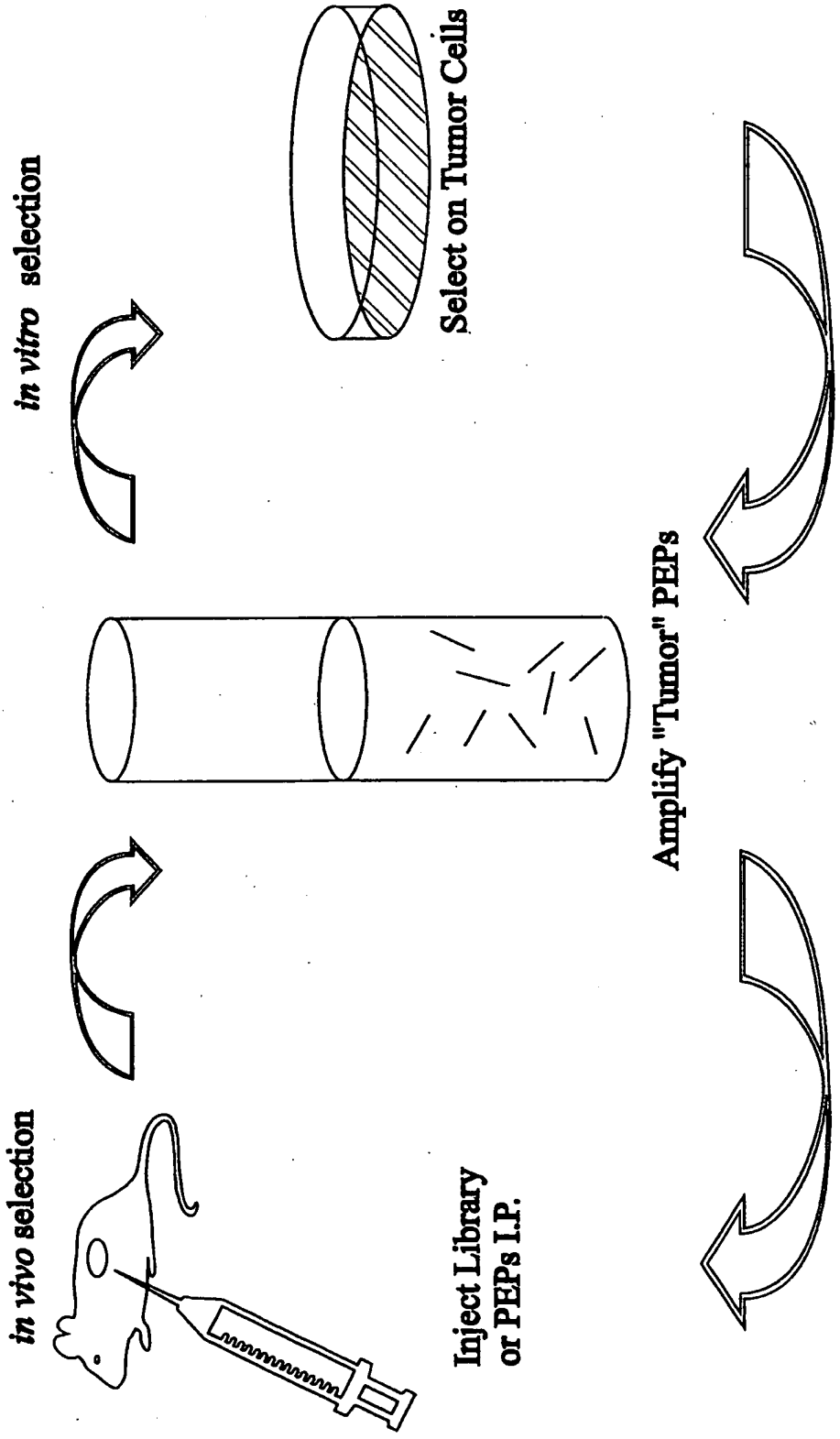
in vivo selection
Inject Library or PEPs I.P.
Repeat as desired
Amplify "Tumor" PEPs
in vitro selection
Select on Tumor Cells

FIG. 1A

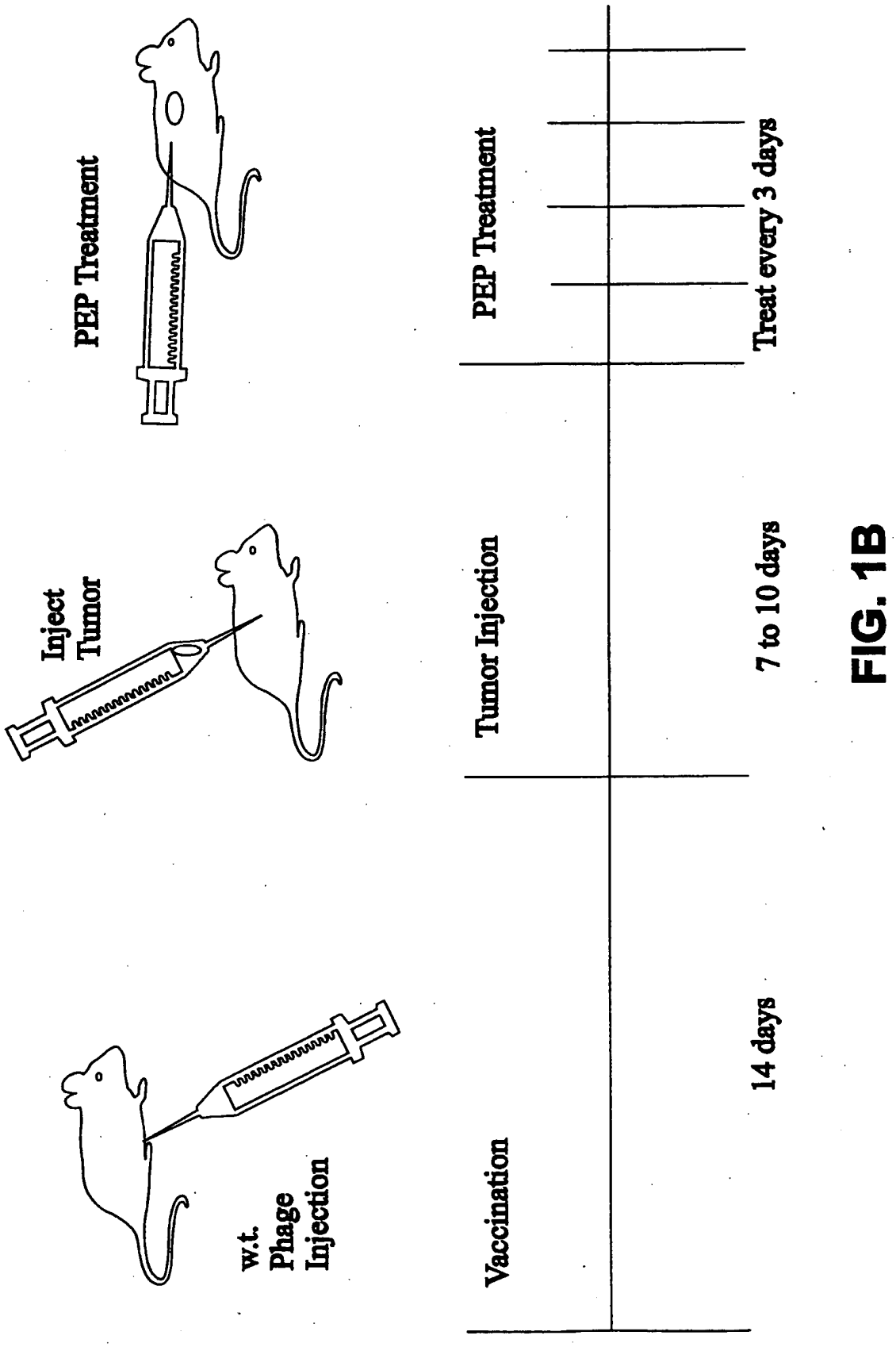
PEP Treatment
Inject Tumor
w.t. Phage Injection
Vaccination
Tumor Injection
PEP Treatment
14 days
7 to 10 days
Treat every 3 days

FIG. 1B

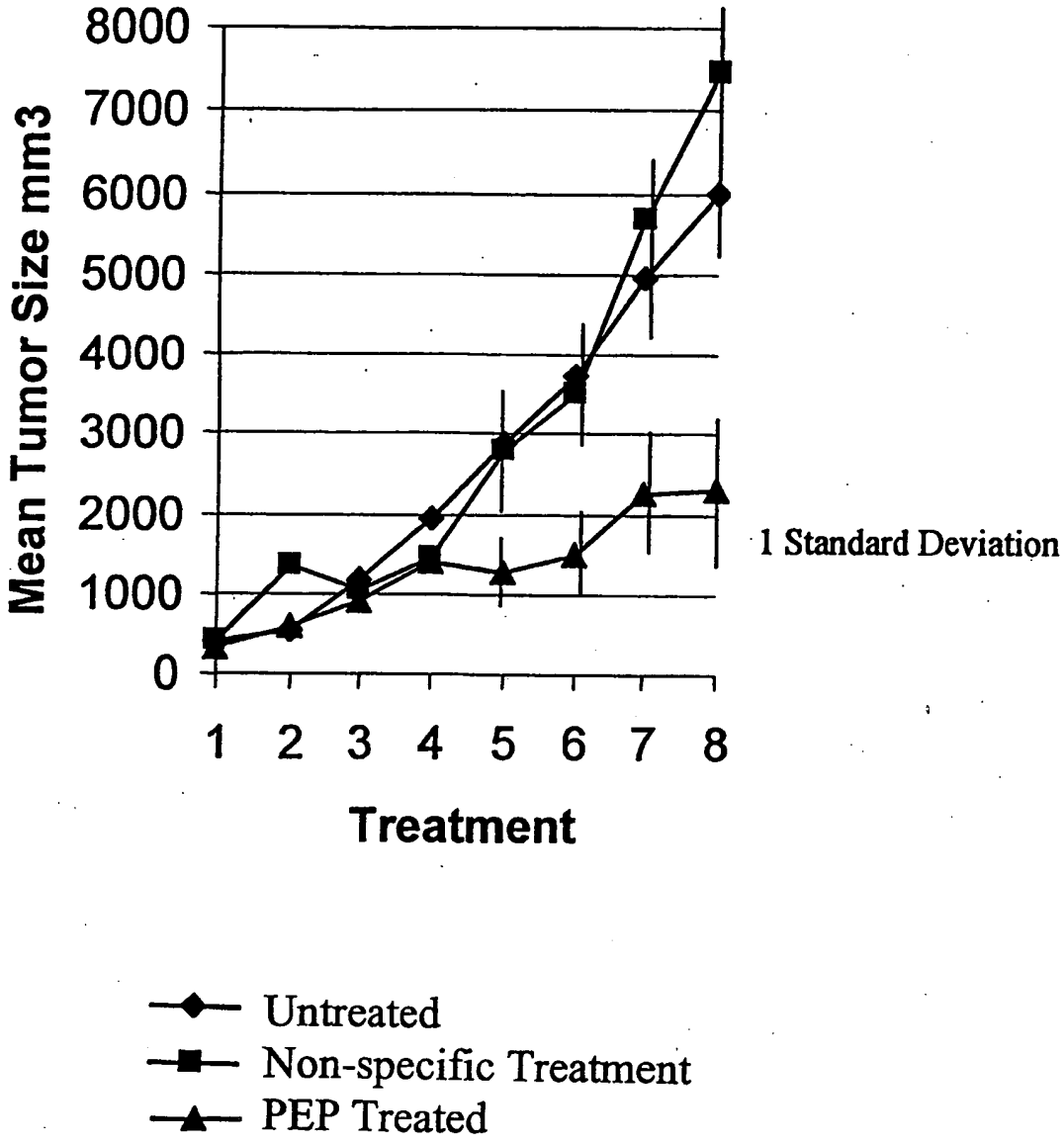
8000
7000
6000
5000
4000
3000
2000
1000
0
Mean Tumor Size mm3
1 2 3 4 5 6 7 8
Treatment
1 Standard Deviation
Untreated
Non-specific Treatment
PEP Treated

Fig. 2A

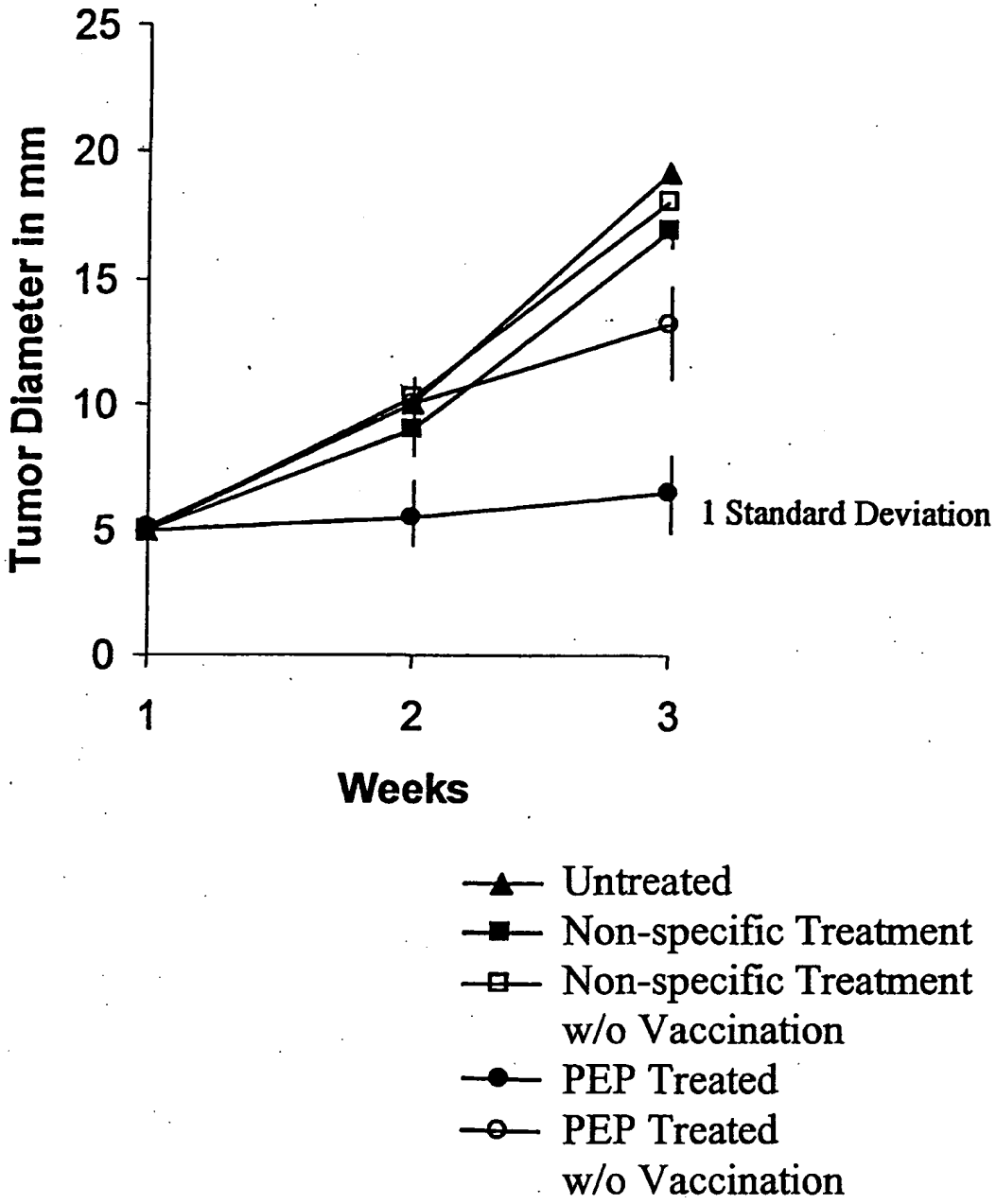
25
20
15
10
5
0
Tumor Diameter in mm
1
2
3
Weeks
1 Standard Deviation
Untreated
Non-specific Treatment
Non-specific Treatment w/o Vaccination
PEP Treated
PEP Treated w/o Vaccination

Fig. 2B

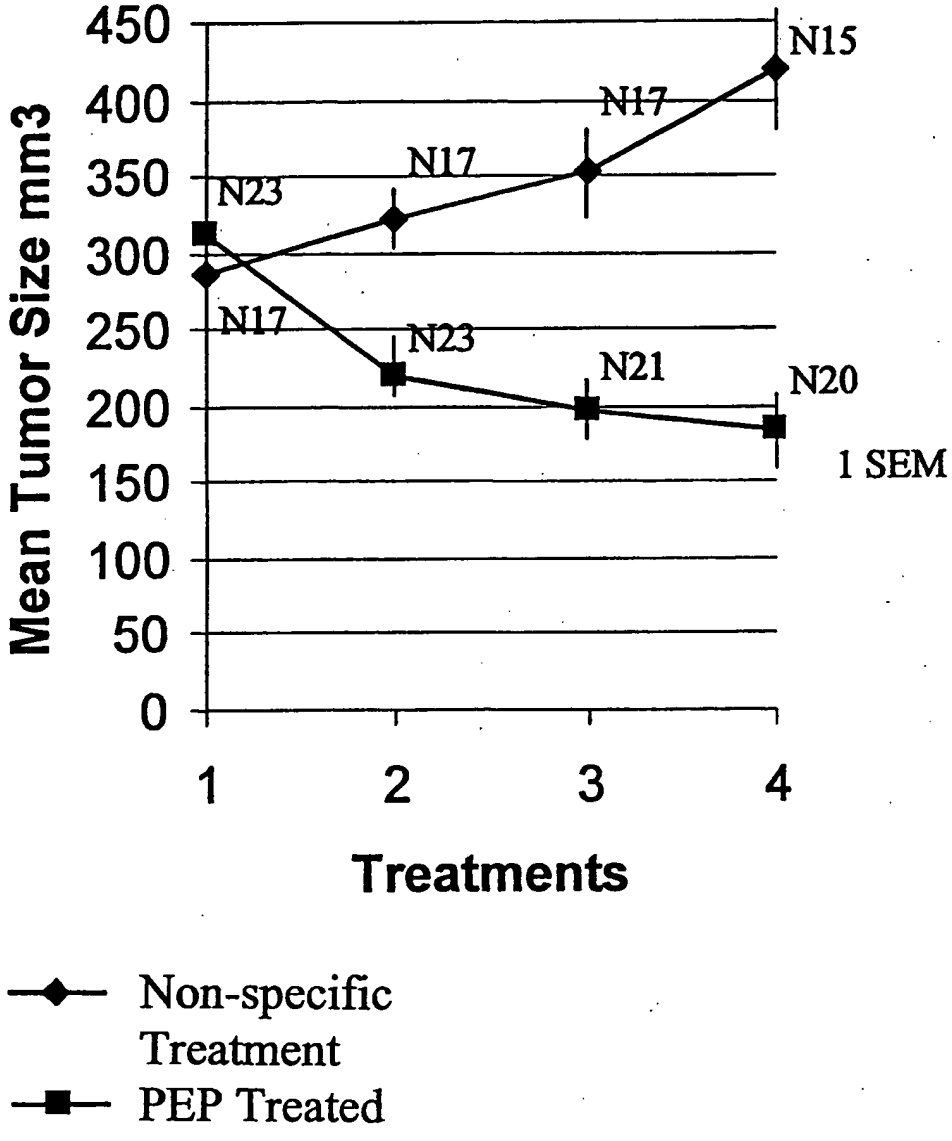
Mean Tumor Size mm3
450
400
350
300
250
200
150
100
50
0
1
2
3
4
Treatments
N15
N17
N17
N23
N17
N23
N21
N20
1 SEM
Non-specific Treatment
PEP Treated

Fig. 3A

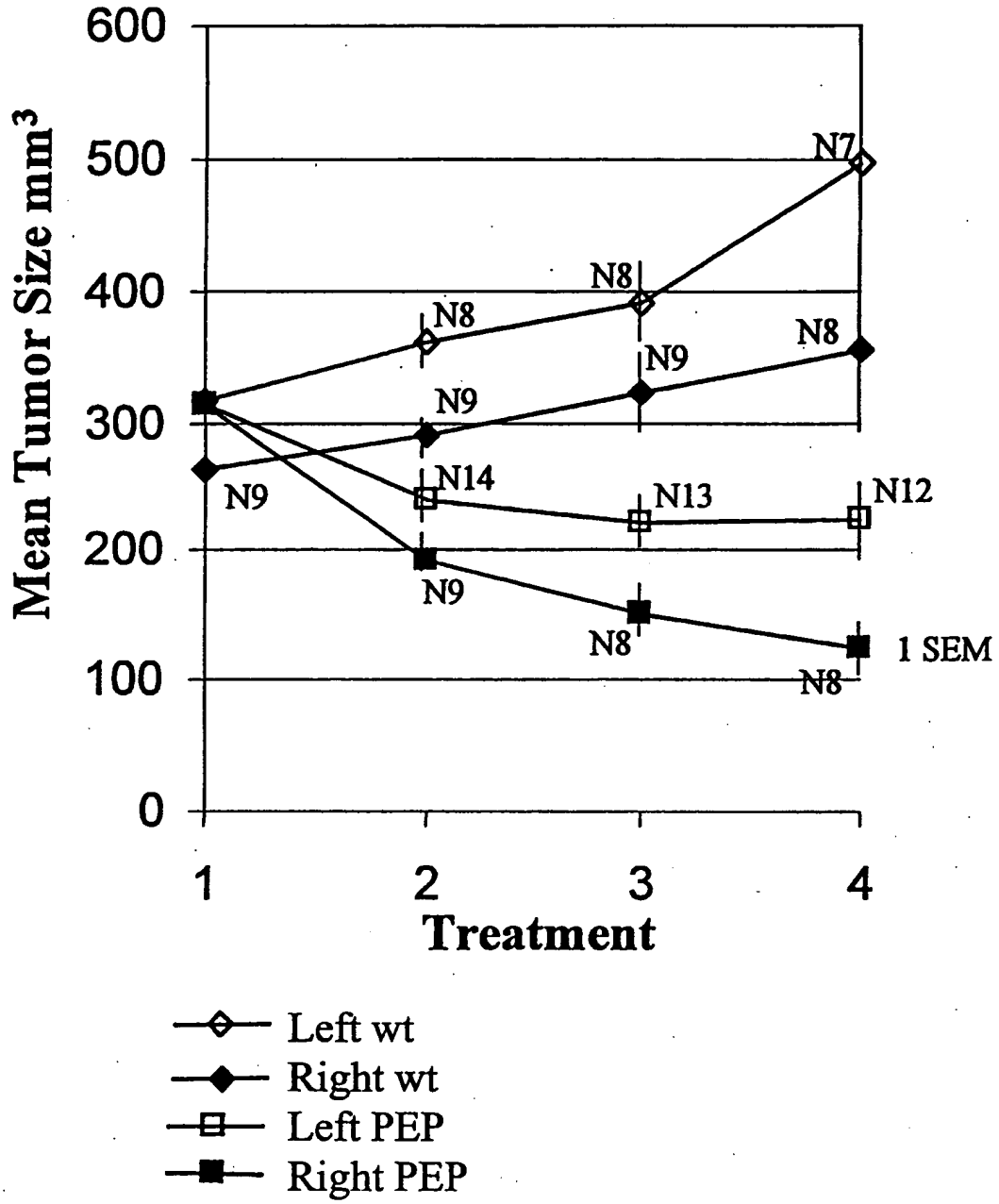
600
500
400
300
200
100
0
Mean Tumor Size mm3
1
2
3
4
Treatment
N7
N8
N8
N8
N9
N9
N9
N14
N13
N12
N9
N8
N8
1 SEM
Left wt
Right wt
Left PEP
Right PEP

Fig. 3B

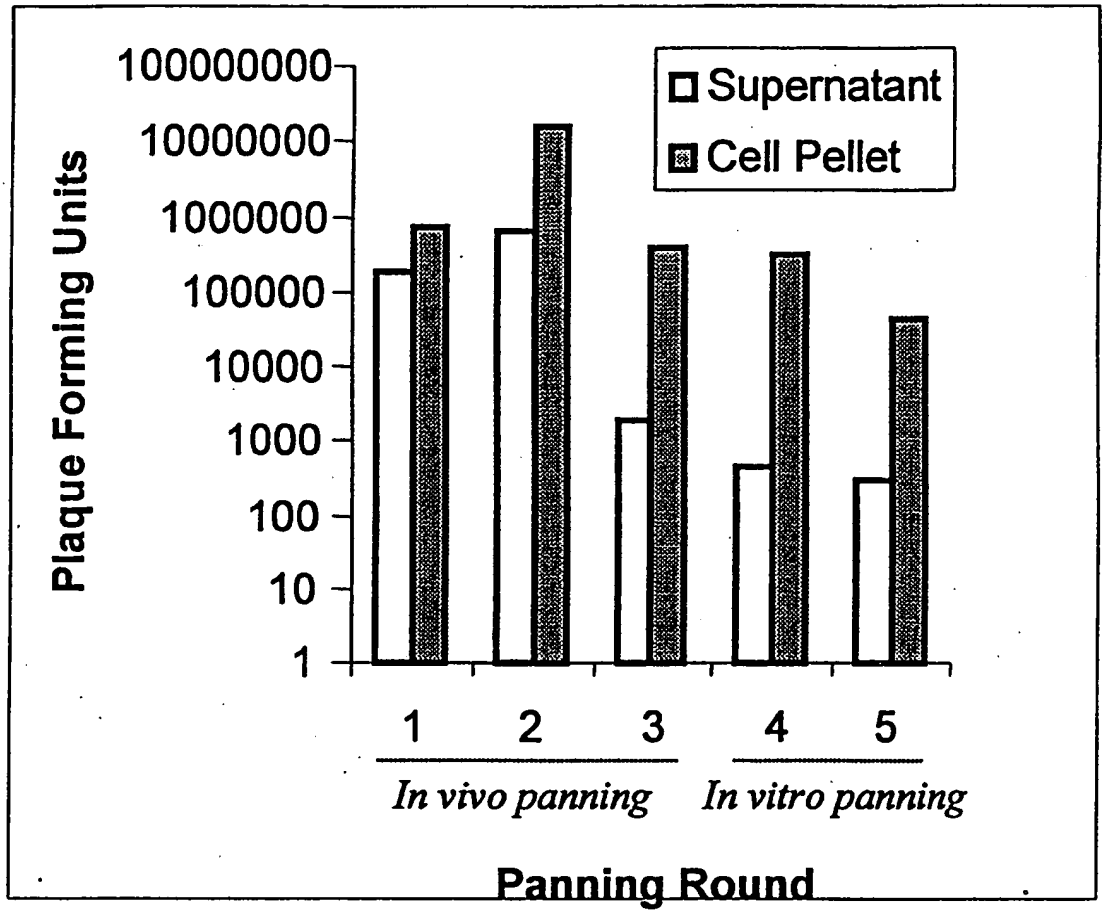
Plaque Forming Units
100000000
10000000
1000000
100000
10000
1000
100
10
1
Supernatant
Cell Pellet
1
2
3
4
5
In vivo panning
In vitro panning
Panning Round

Fig. 4

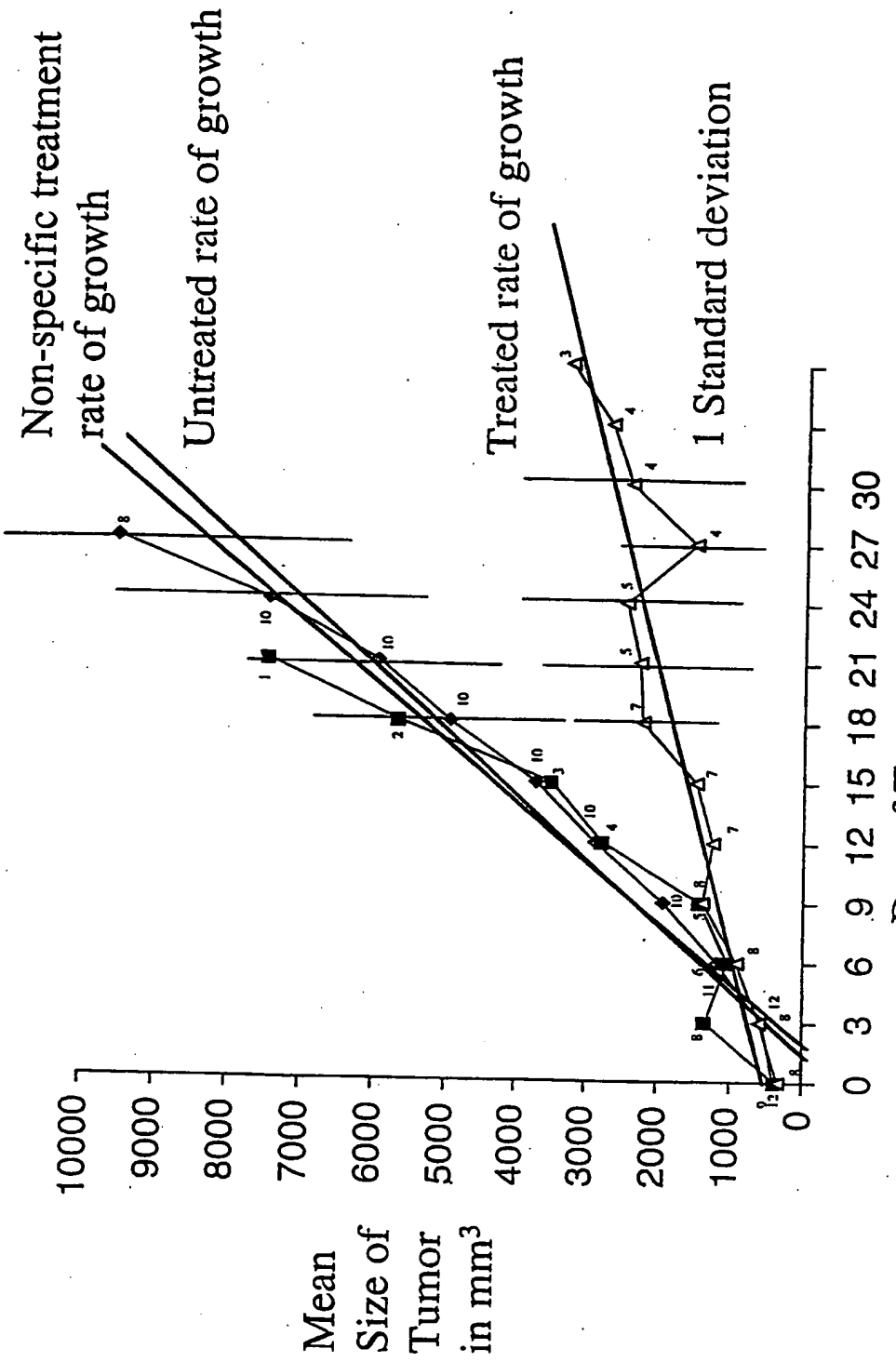
Non-specific treatment rate of growth
Untreated rate of growth
Treated rate of growth
1 Standard deviation
Mean Size of Tumor in mm3
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
0 3 6 9 12 15 18 21 24 27 30
Day of Treatment

Fig. 5A

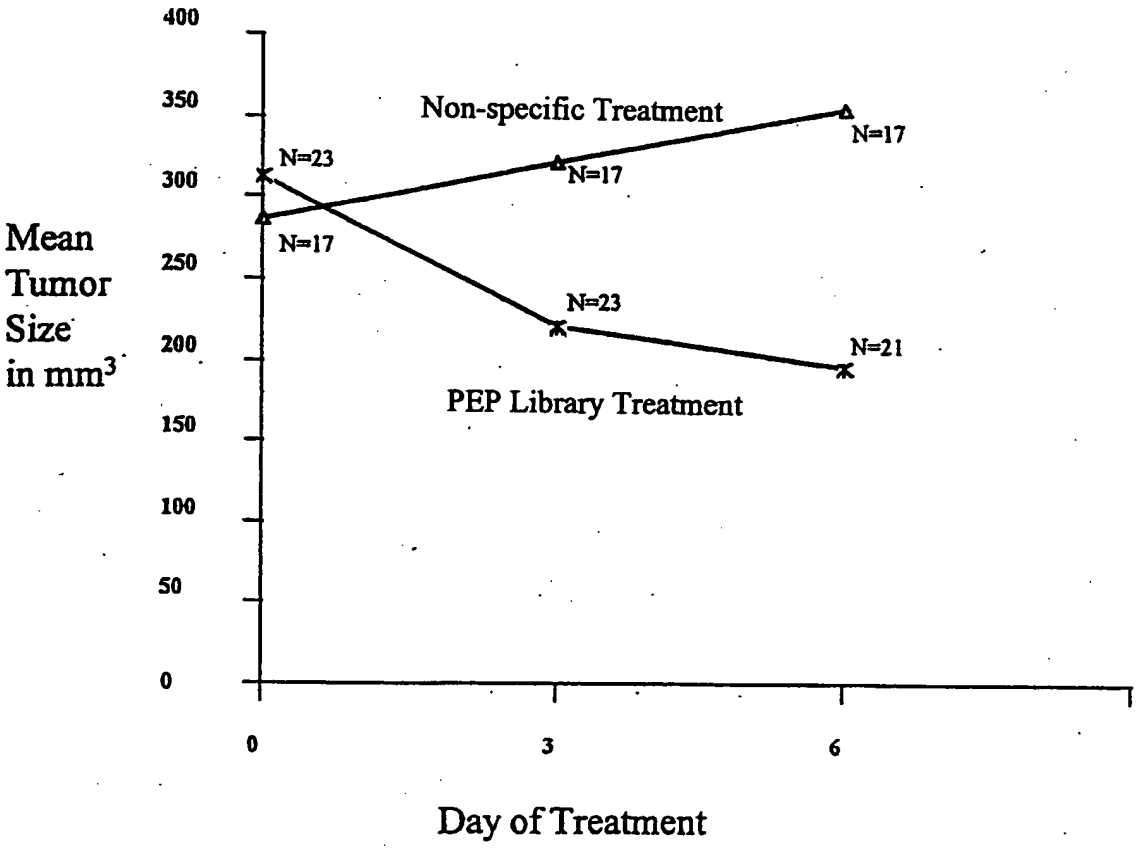
400
350
300
250
200
150
100
50
0
Mean Tumor Size in mm3
Non-specific Treatment
N=23
N=17
N=17
N=17
N=23
N=21
PEP Library Treatment
0
3
6
Day of Treatment

Fig. 5B

Left Side Tumors

PEP Treated

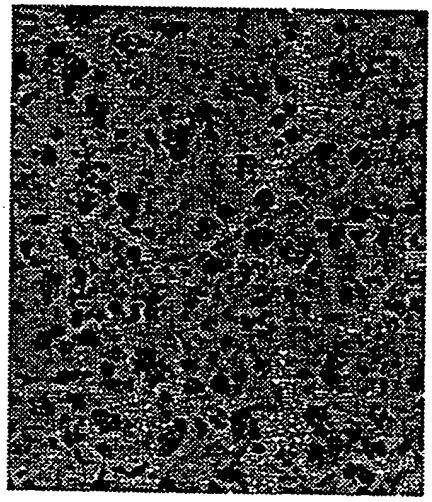

Fig. 6A

Right Side Tumors

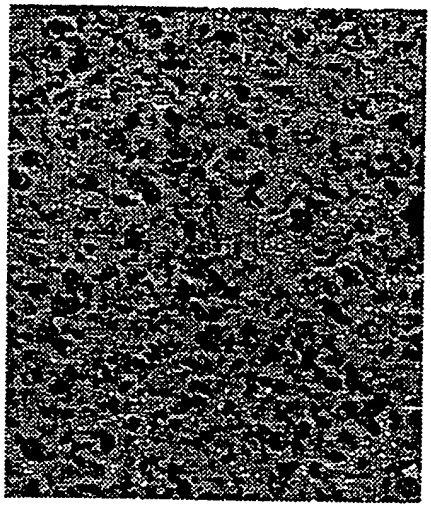

Fig. 6B

wt Treated

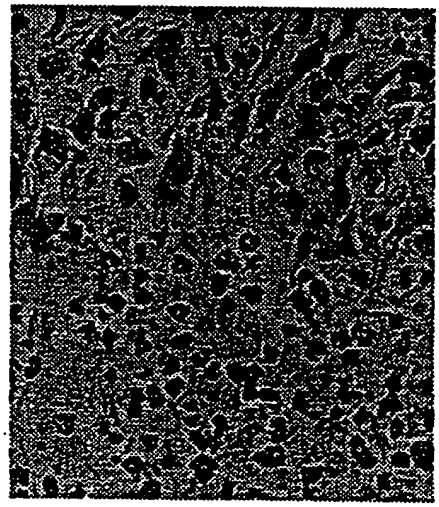

Fig. 6C

Fig. 6D

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/07692

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 436/501, 518; 435/5, 6, 7.1, 7.2, 7.21, 7.23, 7.24, 7.32

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	GALLOP et al. Applications of Combinatorial Technologies to Drug Discovery. 1. Background and Peptide Combinatorial Libraries. J. Med. Chem. 29 April 1994, Vol. 37, No. 9, pages 1233-1251, see entire document, especially Section B pages 1235-1240.	1-29
Y	KREBS et al. Recombinant Human Single Chain Fv Antibodies Recognizing Human Interleukin-6. J. Biol. Chem. 30 January 1998, Vol. 273, No. 5, pages 2858-2865, see entire document, especially pages 2858 and 2860.	1, 2, 4, 8-12, 15, 19, 21, 29, 22, 24-26

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
29 JUNE 1999	05 AUG 1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer MAURIE E. GARCIA
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/07692

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	RENSCHLER et al. Synthetic Peptide Ligands of the Antigen Binding Receptor Induce Programmed Cell Death in a Human B-cell Lymphoma. Proc. Natl. Acad. Sci. USA. April 1994, Vol. 91, pages 3623-3627, see entire document.	1-3, 7-11, 13, 18, 19, 21-25
Y	NUSSBAUM et al. Epitope Location in the Cryptococcus neoformans Capsule is a Determinant of Antibody Efficacy. J. Exp. Med. 17 February 1997, Vol. 185, No. 4, pages 685-694, see page 692.	1, 4, 9-11, 20, 21, 27
Y	WO 94/18345 A1 (AFFYMAX TECHNOLOGIES N.V.) 18 August 1994 (18/08/94), see Abstract; page 23, lines 7-37; page 24, lines 1-16; page 47, lines 11-27; page 51, lines 30-37; page 52, lines 1-14; page 56, lines 33-37 and page 57, lines 1-37.	1-3, 5, 9-11, 13, 16-19, 21-25, 28-29

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/07692

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

C12Q 1/68, 1/70; C12N 15/00; A61K 39/00, 39/395; G01N 33/53, 33/567, 33/574, 33/566, 33/543

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

424/178.1, 184.1; 436/501, 518; 435/5, 6, 7.1, 7.2, 7.21, 7.23, 7.24, 7.32

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, STN (CAPlus, BIOSIS, Medline, Embase, Scisearch)
Search terms: Inventor search, peptide, protein, receptor, combinatorial, library, phage, tumor, neoplastic, inhibition, cell, tissue, IL2 (Interleukin-2), cytokine, FLT3, PI-Ca 9